



Oxidative Stress and Antioxidant Status during Transition Period in Dairy Cows

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ABSTRACT : The study was conducted on 20 Holstein X Sahiwal cross bred dairy cows, with an average milk production of $2,752 \pm 113.79$ liters in 284 ± 5.75 days during a single lactation, that were divided in to two groups of 10 animals. We investigated the oxidative stress and antioxidant status during the transition period in dairy cows. In this study, plasma level of MDA was considered as an indicator of lipid peroxidation and SOD, catalase, GSH and GSHPx as antioxidants. The lipid peroxidation was significantly ($p < 0.001$) higher in cows during early lactation as compared to the cows in advanced pregnancy. A significant positive correlation ($r = +0.831$, $p < 0.01$) was determined between MDA and catalase in early lactating cows. In early lactating cows, blood glutathione was significantly lower than in advanced pregnant cows. However, early lactating cows showed non-significant negative correlation for all antioxidant enzymes with lipid peroxidation. In conclusion, dairy cows seemed to have more oxidative stress and low antioxidant defense during early lactation or just after parturition than advanced pregnant cows, and this appears to be the reason for their increased susceptibility to production diseases (e.g. mastitis, metritis, retention of fetal membranes etc.) and other health problems. (**Key Words :** Antioxidative Enzymes, Oxidative Stress, Production Diseases, Transition Period, Dairy Cow)

INTRODUCTION

The transition period between late pregnancy and early lactation (also called the periparturient period) certainly is the most interesting stage of the lactation cycle. Although the length of time classified as the transition period has been defined earlier by different authors, but we defined this as last 4 weeks before parturition to first 4 weeks after parturition. As stated by Goff and Horst (1997), "The transition from the pregnant, non-lactating state to the non-pregnant, lactating state is too often a disastrous experience for the cows. Nutrition and management of cows during the transition period has received tremendous interest lately. Periparturient period is especially critical for health and subsequent performance of dairy cows (Shanks et al., 1981). Dairy cattle are more susceptible to a variety of metabolic and infectious diseases during the transition period compared with peak lactation (Sordillo et al., 2007).

Host defense mechanisms can be compromised directly

because of numerous physiological and environmental factors during the transition period. For example, physiological stresses associated with rapid differentiation of secretory parenchyma, intense mammary gland growth, and the onset of copious milk synthesis and secretion are accompanied by a high energy demand and an increased oxygen requirement (Gitto et al., 2002). This increased oxygen demand augments the production of oxygen-derived reactants, collectively termed reactive oxygen species (ROS). Although, oxygen is essential for all aerobic organisms, has been termed the "oxygen paradox". Excessive production of free radicals and concomitant damage at cellular and tissue levels are controlled by cellular antioxidants defense systems. Antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to a target molecules (Halliwell and Gutteridge, 2007). The preventive body antioxidative defense systems can be accomplished by enzymatic (e.g. SOD, GSHPx and Catalase) and non-enzymatic mechanisms (e.g. Vitamin E and Selenium). Excessive production of free radicals and ROS, and/or a decrease in body antioxidant defense, lead to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan et al., 2001). When ROS are produced faster than they can be safely neutralized by antioxidant

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mechanisms, oxidative stress results. Therefore, an imbalance between increased production of ROS and reduced availability of antioxidant defenses near the time of parturition increases oxidative stress and may contribute to periparturient disorders in dairy cows (Waller, 2000; Gitto et al., 2002). Unfortunately, oxidative stress as it is not a classical disease, does not exhibit a specific clinical picture. It has been observed that during the transition period cows can experience oxidative stress (Formigoni et al., 1997; Ronchi et al., 2000), which may contribute to periparturient disorders and may be associated with metabolic diseases (Ronchi et al., 2000). The determination of products of peroxidative damage to macromolecules, and antioxidant substances like glutathione and enzymes (e.g. SOD, GSHPx and Catalase) are useful markers for the oxidative stress and antioxidant status respectively. The oxidative destruction of lipids (lipid peroxidation) is a destructive, self perpetuating chain reaction, releasing malonyl aldehyde (MDA) as the end product. However, as per our knowledge, literature on the oxidative stress and antioxidant status during transition period though replete but in Indian perspective there are meager reports has been figured out so far.

The present study was planned with the view of the possible oxidative stress involved during transition period; it was decided to estimate the levels of MDA and SOD, GSHPx, catalase and GSH as an index of oxidative stress and antioxidative status respectively, and to see whether any difference existed in these parameters during advanced pregnancy and early lactation.

MATERIAL AND METHODS

Study design and animals

The study was conducted in the month of December, 2008, at organized dairy farms of northern and central India. Twenty Holstein X Sahiwal cross bred dairy cows aged from 26 months to 7 years and in 1 to 5 parity with an average milk production $2,752 \pm 113.79$ liters in 284 ± 5.75 days of corresponding lactation, were used in the study. Twenty animals were divided randomly into two groups A and B, each group contain 10 animals. Group A cows were in last 4 weeks of pregnancy i.e. advanced pregnancy stage, while group B cows were in first 4 weeks of lactation i.e. early lactation stage. All the cows were tie stall feeder and maintained on concrete floor. All the cows were examined for mastitis before collection of blood samples and selected only mastitis free cows in the study.

Collection of blood samples

Five ml blood sample were taken from jugular vein using heparinized capped 5 ml vials and immediately placed in an ice bath, where they were stored until they were processed, within approximately 2 h from withdrawal. The

blood samples were collected from all cows in the morning on the same day.

Biochemical analysis

All analyses were done within 2 h after sample collection. Extent of lipid peroxidation was estimated in 33% of erythrocyte lysate as the concentration of thiobarbituric acid reactive product malondialdehyde (MDA) by the method of Ohkawa et al. (1979). The values of lipid peroxidation were expressed as (nm) nano moles of MDA produced/g Hb/h using a molar extinction coefficient of pure MDA as 1.56×10^5 . The plasma catalase (CAT) activity was measured as per method described by the Aebi (1983). In brief 20 μ l of 1% erythrocyte lysate was incubated in 1.0 ml of 30 mM H_2O_2 at 37°C and decrease in absorbance was noted every 10 sec interval for one min. at 240 nm in a UV spectrophotometer (Schimadzu UV-1208 UV-VIS, Japan). The catalase activity was expressed as μ moles of H_2O_2 decomposed/min/mg Hb using 36 as molar extinction coefficient of H_2O_2 . The activity of superoxide dismutase (SOD) in 1% erythrocyte lysate was determined by the method of Marklund and Marklund (1974). The assay is based on the ability of SOD to exhibit the autooxidation of pyrogallol in presence of EDTA. The values were expressed as units/mg Hb. The blood glutathione (GSH) levels were measured spectrophotometrically by using method described by the Beutler et al. (1963) and hemoglobin (Hb) by Cyanomethoemoglobin method as described by Gowenlock (1996). Changes in the optical density after reaction with DTNB reagent was estimated and final values were extrapolated on the standard curve of glutathione and GSH concentration expressed as μ g/ml. The plasma glutathione peroxidase (GSH-Px) activity was determined according to the method of Hefeman et al. (1974). The rate of oxidation of GSH by H_2O_2 was used as measure of GSHPx activity and expressed as units/mg Hb.

Statistical analysis

All data's were analysed by using Student's t-test to know the significance values between the groups. Pearson's correlations between level of peroxidation and activity of antioxidative enzymes were calculated and, where significant, are reported in the text. All statistical parameters were calculated as per the Snedecor and Cochran (1994).

RESULTS

Oxidative stress

Changes in the oxidative stress and antioxidant status in both the groups has been presented (Tables 1 and 2). The lipid peroxidation (plasma MDA concentration) was significantly ($p < 0.001$) higher in early lactating cows

Table 1. Oxidative stress and antioxidant status in advanced pregnant (group A) and early lactating cows (group B) (Values are expressed as mean±SE)

Group	No. of animals	MDA	Cat	SOD	GSH	GSHPx	Hb
A	10	7.59±0.72	42.52±6.98	6.99±0.45	71.81±11.41	1.93±0.22	11.62±0.50
B	10	13.34±2.68**	48.33±6.55	6.37±0.72	28.99±6.58*	1.19±0.13	12.08±0.32

* Denotes significant difference at $p < 0.002$. ** Denotes significant difference at $p < 0.001$.

Table 2. Correlation between oxidative stress and antioxidative status in advanced pregnant (group A) and early lactating cows (group B)

Group	No. of Animals	MDA	CAT	SOD	GSH	GSHPx
A	10	1.00	0.831**	-0.155	0.336	-0.330
B	10	1.00	-0.553	-0.231	-0.196	0.350

** Correlation is significant at the 0.01 level (2-tailed).

(group B) as compared to advanced pregnant cows (group A). The mean±SE plasma level of MDA in advanced pregnant dairy cows was 7.59±0.72 nm of MDA produced/g Hb/h, which was nearly double in early lactating cows, 13.34±2.68 nm of MDA produced/g Hb/h. A significant positive correlation ($r = +0.831$, $p < 0.01$) was determined between MDA and catalase in group A cows (Figure 1), while negative correlation ($r = -0.553$) was in group B animals between oxidative peroxidation (MDA) and antioxidative status (CAT).

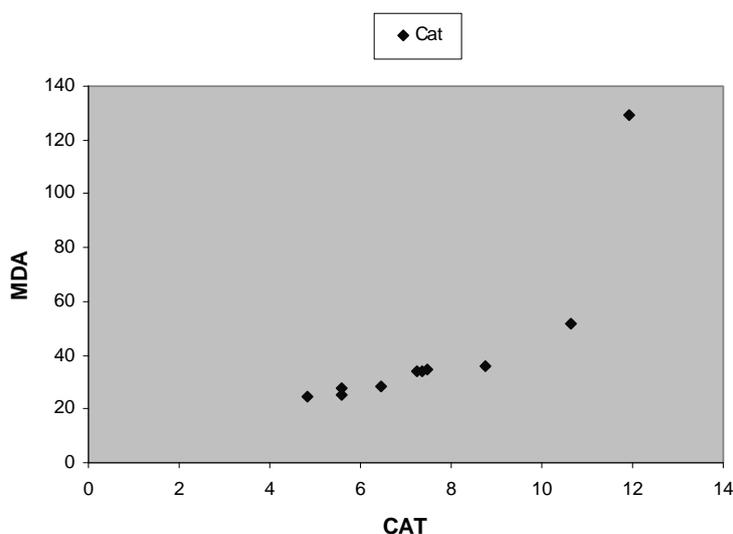
Anti-oxidative status

In early lactating cows, mean±SE of blood glutathione (GSH) was 28.99±6.58 µg/ml, which was significantly lower than that of advanced pregnant cows, 71.81±11.41 (Table 1). The mean±SE of catalase activity was non-significantly higher in early lactating cows. The activities of other anti-oxidative markers like SOD and GSHPx were nearly similar in both the groups during transition period.

A significant positive correlation was detected between MDA production and CAT activity in the animals of group A (Figure 1). The activity of blood GSH was also increased with the increased peroxidation (Table 2), while SOD and GSHPx activity had negative correlation with MDA production of group A cows. In early lactating cows all antioxidant enzymes had non-significant negative correlation with lipid peroxidation (Table 2).

DISCUSSION

The role of antioxidants in health and disease was studied extensively in both human and animal medicine (Valko et al., 2007). Dairy cows undergo massive metabolic adaptations during the onset of lactation, and it was postulated that some of these physiological events may negatively impact the health of the dairy cows (Sordillo et al., 2009). Lipid peroxidation is one of important consequences of oxidative stress (Kumaraguruparan et al., 2002). The determination of lipid peroxidation products

**Figure 1.** Showing the positive correlation between MDA and CAT during advanced pregnancy.

allows for the estimation of the intensity of this process; moreover, it can be used for the evaluation of oxidative stress severity (Halliwell and Whiteman, 2004). Lipids are the most susceptible for peroxidative damage due to low energy necessary for the initiation of the process as well as the presence of unsaturated bonds (Balasinska, 2004).

In the present study lipid peroxidation (plasma MDA production) was significantly ($p < 0.001$) higher in early lactating cows than advanced pregnant cows, which was nearly double. The findings of our study are in corroboration with the reports of Saleh et al. (2007); they used thiobarbituric acid reactive substances (TBARS) values as a marker of lipid peroxidation in cattle. Oxidative stress in cows is a contributory factor to increase disease susceptibility (Sordillo, 2005), since metabolic demands associated with late pregnancy, parturition and initiation of lactation would be expected to increase the production of reactive oxygen species (ROS), resulting oxidative stress. A relationship between oxidative stress (lipid peroxidation) and antioxidant status (catalase) was found significantly positive in advanced pregnant cows, while non-significant negative correlation was found in early lactating cows. Saleh et al. (2007) have also been reported that depletion of antioxidant activity and increase oxidative stress during periparturient period that simulates our results. Stress due to calving has a greater effect on this imbalance in addition to decreased feed intake and plasma level of important nutritional antioxidants/co-factors due to increased demand of minerals and vitamins during advanced pregnancy and more drainage in the colostrum. Unfortunately, it has not been well known that how oxidative stress can affect health and well-being, particularly during the times of high metabolic activity. The performance of high yielding dairy cattles can be optimized to a certain extent by supplementing diets with optimal levels of micronutrients with antioxidant capabilities. However, oxidative stress continues to be a problem in transition cows. Innovative approaches to combat the progression of stress and to enhance the antioxidant defense mechanisms of dairy cattle during times of increased metabolic demands seems to be pertinent (Sordillo and Aitken, 2009).

In the present findings, blood GSH was significantly lower in early lactating cows than that of advanced pregnant cows. In contrast, catalase activity was not significantly higher in early lactating cows. However, Aitken et al. (2009) had reported that activities of glutathione peroxidase (GSHPx1 and GSHPx4) were increased during early lactation. The activities of other anti-oxidative markers like SOD and GSHPx were nearly similar in both the groups during transition period during the study. Holbrook and Hicks (1978) also observed no significant differences in the SOD concentration throughout the lactation of nonmastitic cows.

The activity of blood GSH was increased with the increased lipid peroxidation, while SOD and GSHPx activity had negative correlation with MDA production in group A cows. Therefore, an imbalance between increased production of ROS and reduced availability of antioxidant defense near the time of parturition might increase oxidative stress and may contribute to periparturient disorders in dairy cows (Waller, 2000; Gitto et al., 2002). Increased incidence of disease during the periparturient period is related directly to numerous genetic, physiological, and environmental factors that can compromise the cow's immunological defenses (Sordillo, 2005). A relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential was established in both humans and dairy cows (Bernabucci et al., 2005; Sordillo et al., 2007). The possibility that oxidative stress during the transition period particularly during parturition may be a major underlying cause of inflammatory and immune dysfunction in dairy cattle has earlier been supported by various studies conducted either *in vivo* or *in vitro* (Sordillo and Aitken, 2009).

Superoxide dismutase, catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen. Hydrogen peroxide degrades further to water by other antioxidant enzymes, such as glutathione peroxidase and catalase. In mammalian cells, there are three types of superoxide dismutase: cytosolic CuZn superoxide dismutase (CuZnSOD), mitochondrial manganese superoxide dismutase (MnSOD), and extracellular superoxide dismutase (ECSOD). Glutathione peroxidase proteins catalyze the reduction of organic hydroperoxides, lipid peroxides, and hydrogen peroxide, using glutathione as the reducing agent, thereby also protecting cells from oxidative damage resulting from normal oxidative metabolism. There are four known GSHPx that contain selenocysteine at the active site.

In early lactating cows all antioxidant enzymes had non-significant negative correlation with lipid peroxidation. During advanced pregnancy and early lactation increased demand of micronutrients do not usually full fill the resulting deficiencies occurring due to natural protective substances or excess exposure to stimulators of "reactive oxygen metabolites" (ROM), and this might results in high lipid peroxidation and decreased level of antioxidant enzymes. Significant correlation between antioxidant supplementation and decreased incidence of mastitis (Weiss et al., 1997; Allison and Laven, 2000) has been reported previous, which supports our results and presumptions. Decreased level of antioxidant enzymes during early lactation in our study could be supported as oxidative stress increases during parturition and early lactation stages. Oxidative stress increase causes continuous increase in the concentration of lipid peroxidation products and decrease in the level of enzymatic and non-enzymatic antioxidants after

transiently increased activity to combat the toxic effects of reactive oxygen species (ROS). The antioxidant enzymes such as GSHPx and catalase might have important functions in alleviating the toxic effects of ROS (Vannucchi et al., 1997; Kale et al., 1999). Infection and tissue repair are common even in well-managed dairy herds, and cows may experience some degree of immune response, especially after calving. Stress disease and induction of the immune response increases nutrient requirements (Madsen et al., 1990; Madsen, 1991) has been known. Inadequacies of these nutrients required for both immunity and antioxidant defense could impair function of both systems. To optimize performance, oxidative stress in high producing cows must be controlled by supplying all known antioxidant nutrients and by minimizing effects of substances that stimulate ROS. Significant correlation between antioxidant supplementation and decreased incidence of mastitis (Weiss et al., 1997; Allison and Laven, 2000) has already been reported. Conclusively, dairy cows seemed to have more oxidative stress and low antioxidant defense during early lactation or just after parturition than advanced pregnant cows, and this seemed to be the probable reason for their increased susceptibility to production diseases (e.g. mastitis, metritis, retention of fetal membranes etc.) and other health problems.

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